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Assessment of Biochemical Parameters, Kidney Function, and Long-term Outcome in Renal Transplant Recipients

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Abstract

Background and Aims: New-onset of diabetes after transplantation (NODAT) is the most significant complications arising post-renal transplantation and affecting the long-term graft outcome and recipient survival. Assessment of renal function in kidney transplant recipients might help in understanding the better outcome of the graft and also the factors associated with NODAT. The present study was aimed to estimate the biochemical parameters, electrolytes, and minerals in the serum among renal transplant recipients and healthy controls (HC) and to evaluate the graft function, graft outcome and patient survival. **Materials and Methods:** Biochemical parameters (creatinine, urea, and uric acid), electrolytes (sodium, potassium, and chloride), and minerals (calcium and phosphorus) were estimated in serum by enzymatic method using commercially available kits in 100 HC, 80 NODAT, and 80 Non-NODAT subjects. The graft outcome was assessed by comparing serum creatinine levels and urinary creatinine clearance at 0 month and 60 months. The survival rate was evaluated by Kaplan-Meier survival curve. **Results:** The mean age was significantly higher in NODAT versus non-NODAT at $P < 0.0009$. Significant gender difference was observed in NODAT and non-NODAT versus HC at $P < 0.0001$. The levels of creatinine, urea, and uric acid were significantly more in NODAT versus HC at $P < 0.0001$, $P < 0.0001$, and $P < 0.006$. The mean levels of sodium and phosphorus were significantly lower in NODAT versus HC at $P < 0.008$ and $P < 0.029$. In multinomial logistic regression analysis, age, male gender, creatinine, and urea significantly predicted the outcome and the Receiver Operating Characteristic analysis revealed creatinine to be better marker for assessing kidney function. The Kaplan-Meier survival curve analysis showed decreased survival rates in NODAT than non-NODAT. **Conclusion:** Older age (above 40), hyponatremia, and hypophosphatemia could be significant risk factors for NODAT development.

Keywords: Biochemical parameters, Graft outcome, Kidney function, New-onset of diabetes after transplantation, Renal transplant recipients, Survival rate

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Introduction

Renal transplantation has been conducted worldwide and considered as the best treatment of choice for End Stage Renal Disease. With better transplantation techniques and new immunosuppressive drugs, patients' survival rates have increased, but also have many medical complications leading to graft loss, comorbid conditions, or even patients' loss, if untreated.[1] There are many complications in the initial and late post-operative duration, which are responsible for increased comorbidities and poor standard of life in renal transplant recipients,[2] including acute allograft dysfunction, delayed graft function, unexpected side effects of higher dosages of drugs, and infections. Nevertheless, persistent transplant-related problems are more significant, which include various infections, hypertension and cardiovascular diseases (CVDs), bone disease, cataracts, post-transplant erythrocytosis, chronic rejection, cancer, recurrent diseases, and the most significantly new-onset of diabetes after transplantation (NODAT).

NODAT is the occurrence of diabetes post transplantation of any organ, affecting people who do not have previous history of diabetes.[3] NODAT is one among the main problem in the life-long survival of the graft and recipient[4] and also related to greater risk of CVDs, affecting survival of the graft, rejection and loss leading to graft failure infections, mortality.[5,6] and increased health care costs.[7] Therefore, assessment and management of kidney function are essential in renal transplant recipients for better graft outcome. Kidney function can be assessed by estimating various parameters such as creatinine, blood urea nitrogen, uric acid, and several electrolytes.[8,9]

Creatinine is the metabolite of dietary meat and creatine phosphate which is mainly present in skeletal muscle and its production in the body is directly proportional to muscle mass.[10] Some studies have reported the association of lower serum creatinine with increased risk of dysglycemia and Type 2 diabetes mellitus (T2DM).[11,12] Earlier studies have demonstrated that elevated blood urea nitrogen levels are related to increased risk of diabetes mellitus (DM) and insulin use.[13,14] Serum uric acid is the end product of the nucleotides, purine and its excess secretion, and reduced elimination by kidneys result in higher uric acid levels in individuals. Elevated uric acid levels in blood may cause gout and they are found to be associated with various ailments, such as diabetes, CVDs, metabolic syndrome, and kidney dysfunction.[15] Earlier studies have shown positive correlation of

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augmented serum uric acid with diabetes,[16,17] while some studies have not shown any relationship[18,19] or some showed opposite correlation.[20-22]

Micronutrients, such as minerals and trace elements, are important for proper physiological functions of the body;[23] deficiency of these might cause diseases[24] and are directly linked to DM.[25] The key components of micronutrients include vitamins, macro elements, organic acids, and trace elements. The major constituents of macro elements comprise electrolytes including sodium, potassium, chlorides, and minerals such as calcium, phosphorus, iron and magnesium, and trace elements consisting of boron, cobalt, copper, chromium, iodine, sulfur, molybdenum, and zinc which stimulate the action of insulin through action of insulin receptor sites[26] and have a major role in the pathogenesis and development of T2DM through altered mode of action.[27]

As the pathophysiological mechanism of NODAT is identical to T2DM,[28] the present study aimed to estimate the levels of creatinine, urea, uric acid, sodium, potassium, chloride, calcium, and phosphorus in serum among renal transplant recipients and healthy controls (HC) to assess the kidney function and also to understand their association with NODAT. We followed up the patients for 5 years to evaluate the graft function, graft outcome, and patient survival.

Materials and Methods

Subjects

The present study was carried out during the period 2014–2019 and the subjects who attended Kidney Transplant Unit of Mahavir Hospital and Research Centre and Transimmun Diagnostics (Irrum Manzil), Hyderabad were enrolled into study. In the present study, 260 subjects were enrolled, from which 100 were HC, 80 were NODAT, and 80 were non-NODAT. Patients were categorized based on the onset of diabetes after renal transplantation. Demographic features such as age, sex, height, and weight were recorded and Body Mass Index (BMI) was calculated for all the subjects.

- Patients who developed diabetes after renal transplantation (NODAT) group – They were patients who developed diabetes after renal transplantation and without any signs of hyperglycemia before renal transplantation.
- Patients without diabetes after renal transplantation (non-NODAT) group – They were the patients who did not develop diabetes after renal transplantation and without any history of diabetes before renal transplantation.
- HC – without any history of diabetes or any other diseases. The donors of NODAT and non-NODAT subjects were taken as HC.

Ethical Clearance

Institutional Ethical Committee of Bhagwan Mahavir Medical Research Centre reviewed and approved the protocol. Informed consents, personal history, and clinical details were acquired from all the subjects participated in the study.

Inclusion, Exclusion Criteria, and Sample Collection

Kidney transplant recipients (KTRs) without prior history of diabetes, their donors, and patients who were willing to give the consent were included in the study. Patients <18 years and more than 60 years, presence of secondary immunodeficiency diseases like Human Immunodeficiency Virus, malignancy, cardiac disease and pregnant women, and patients unwilling to comply with the study were excluded from the study. Approximately 5 ml of blood was drawn from NODAT, non-NODAT, and HC subjects. The blood was collected in clot activator tubes for serum separation and estimation of biochemical parameters.

Methodology

Estimation of biochemcial parameters

Biochemical parameters including renal function tests (creatinine, urea, and uric acid), electrolytes (sodium, potassium, and chloride), and minerals (calcium and phosphorus) were estimated in serum by enzymatic method using commercially available kits (Agappe Diagnostics Ltd.) in Merck, Semi-auto Analyzer. Creatinine was estimated by Jaffe's method (Kinetic and End Point Method). The expected range for serum creatinine was taken as 0.9–1.5 mg/dl and 0.8–1.3 mg/dl for male and female, respectively. Urea was estimated by urease or GLDH methodology and the normal range for urea in serum was taken as 0–50 mg/dl. Uric acid was assessed by uricase methodology and the normal range for serum uric acid was considered as 3.4–7.0 mg/dl for men and 2.4–5.7 mg/dl for women. Sodium, potassium, and chloride were estimated by Electrolytes Test Kit (Excel Diagnostics Pvt Ltd). The reference ranges for sodium, potassium, and chloride were taken as 135–155 mEq/l, 3.5–5.5 mEq/l, and 97–108 mEq/l. Calcium was estimated by modified OCPC methodology and the normal range for calcium in serum was taken as 8.8–10.2 mg/dl. Phosphorous was estimated by phosphomolybdate methodology and the reference range was taken as 2.5–4.5 mg/dl.

Long-term outcome, graft survival, and patient survival analysis

The renal transplant recipients, 80 each from NODAT and non-NODAT groups, were followed for 5 years (60 months) to determine the influence of NODAT on the long-term outcome, graft, and patient survival. Creatinine levels in serum were estimated at 0 month (start of the study) and 60 months (end of the study) and urinary creatinine clearance (CrCl) was calculated to analyze the graft outcome of the recipients. Rate of infections was also accounted in the patients during the study. The survival rate of the recipients was also assessed.

CrCl was calculated from creatinine levels of serum using the Cockcroft-Gault formula, which determines CrCl using age, gender, and weight (in kg) of the patients. In female, the resulting CrCl is multiplied by 0.85 to adjust the lower CrCl in females,[29] as shown in formula below:

CrCl = (140-Age) × Weight (kg) × (0.85 in case of female)/(72 × serum creatinine [mg/dl]).

Statistical Analysis

Demographic features and biochemical parameters were expressed as mean ± SD and the difference among the groups were calculated by Student's *t*-test for continuous variable and Chi-squared test (χ^2 test) for categorical variables. GraphPad prism version 5.0 was used to calculate the test of significance.

Statistical differences between the groups were computed by Mann–Whitney U test (non-parametric). One-way analysis of variance (ANOVA) was performed to relate the variances between the means of the variables among the groups and *post hoc* test was executed for multiple comparisons using Dunnett T3 to know the difference between specific groups using IBM SPSS statistical software program version 20.0. Multinomial logistic regression (MLR) analysis was performed to predict the outcome and Receiver Operating Characteristic (ROC) curve analysis was performed and area under the curve (AUC) was obtained for each marker using SPSS to know the best diagnostic marker. Kaplan-Meier survival analysis curve was assessed by SPSS to calculate the patient survival rate. Differences at $P \leq 0.05$ were considered to be significant.

Results

Demographic Features in HC, NODAT, and Non-NODAT

A total of 260 subjects (HC [n = 100], NODAT [n = 80] and non-NODAT [n = 80]) were enrolled into the study. Demographic features such as gender, age, and BMI were analyzed in the subjects and shown in Table 1. The mean age (in years) was found to be 39.83 ± 10.21, 34.78 ± 8.59, and 42.56 ± 7.27 in NODAT, non-NODAT, and HC. Significance was found in non-NODAT versus HC at $P < 0.0001$ as well with NODAT at $P < 0.0009$. Significance was not observed in NODAT compared to HC. We observed majority of NODAT and HC subjects to be in 40–60 years age group while most of the non-NODAT patients were found to be below 40 years [Figure 1].

The frequency of males [63 (78.75%) and 67 (83.75%)] was found to be high when compared to females [17 (21.25%) and 13 (16.25%)] both in NODAT and non-NODAT, respectively, while in HC, the frequency of females (66 [66%]) was observed to be more as compared to males (34 [34%]). Significant gender difference was observed in NODAT and non-NODAT in comparison with HC at $P < 0.0001$. Significance was not observed in NODAT compared to non-NODAT [Figure 2]

The mean BMI (in kg/m^2) was found to be 23.31 ± 3.03, 23.69 ± 2.98, and 24.00 ± 1.92 in NODAT, non-NODAT, and HC. Significance was not observed in NODAT and non-NODAT when compared to HC. In the present study, the most of the subjects were observed to be of normal weight in HC, NODAT, and non-NODAT groups. In HC, 65 (65%) subjects were of normal weight and 35 (35%) subjects were observed to overweight. None of the subjects were found to be underweight in HC. In NODAT, 48 (60%) subjects were found to be of normal weight, followed by 25 (31.25%) to be overweight and 7 (8.75%) to be underweight. In non-NODAT, 50 (62.5%) subjects were observed to be of normal weight, followed by 27 (33.75%) were overweight and 3 (3.75%) were underweight. Obese (>30 kg/m^2 BMI) subjects were not observed in any of these groups [Figure 3].

Biochemical Parameters in HC, NODAT, and Non-NODAT

Biochemical parameters such as creatinine, urea, uric acid, sodium, potassium, chloride, calcium, and phosphorus were estimated in serum in 100 HC, 80 NODAT, and 80 non-NODAT subjects, shown in Table 2.

The mean creatinine levels (in mg/dl) were found to be significantly more in NODAT (1.53 ± 0.43) and non-NODAT (1.40 ± 0.58) when compared to HC (0.82 ± 0.15) at $P < 0.0001$. Significance was also found in NODAT when compared with non-NODAT at $P < 0.026$. The mean urea levels were found to be significantly more in NODAT (36.39 ± 10.24) and non-NODAT (32.68 ± 12.92) when compared to HC (22.74 ± 4.41) at $P < 0.0001$. Significance was also found in NODAT when compared with non-NODAT at $P < 0.0009$. The mean uric acid levels were observed to be significantly higher in NODAT (5.99 ± 1.36) and non-NODAT (5.87 ± 1.31 when compared to HC (5.41 ± 1.28) at $P < 0.006$ and $P < 0.014$, respectively. Significance was not found in NODAT compared to non-NODAT. The mean levels of sodium (in mEq/l) were lower in NODAT (135.9 ± 3.09) and non-NODAT (136.0 ± 2.03) as compared to HC (136.4 ± 1.90). Significance was observed in NODAT versus HC at $P < 0.008$ but not in non-NODAT versus HC. The mean levels of potassium (in mEq/l) were 4.20 ± 0.46, 4.22 ± 0.73, and 4.09 ± 0.54 in HC, NODAT, and non-NODAT, respectively, and the results were comparable between the groups. The mean levels of chloride (in mEq/l) in HC, NODAT, and non-NODAT were 100.4 ± 2.76, 99.26 ± 6.74, and 100.5 ± 2.6, respectively, and significance was not observed between the groups. The mean levels of calcium (in mg/dl) were observed to be 8.82 ± 0.33, 8.81 ± 0.46, and 8.84 ± 0.42 in HC, NODAT, and non-NODAT, respectively, and the levels were comparable between the groups. The phosphorus levels (in mg/dl) were observed to be lower in NODAT (4.60 ± 0.69) and non-NODAT (4.74 ± 1.04) as compared to HC (4.84 ± 0.70), and significance was found between NODAT versus HC but not with non-NODAT versus HC [Figure 4a and b].

One-way ANOVA in HC, NODAT, and Non-NODAT

A one-way ANOVA was executed to relate the variances between the means of the variables among the groups HC, NODAT, and non-NODAT. In the present study, age, gender, creatinine, urea, and uric acid were observed to be significant among the groups at $P < 0.05$ for the three conditions ($F[2, 257] = 18.122$, $P < 0.0001$), ($F[2, 257] = 36.244$, $P < 0.0001$), ($F[2, 257] = 78.071$, $P < 0.0001$), ($F[2, 257] = 50.051$, $P < 0.0001$), and ($F[2, 257] = 4.937$, $P < 0.008$), respectively. *Post-hoc* test for multiple comparisons using Dunnett T3 and Tukey

Table 1: Demographic and biological characteristics in HC, NODAT, and Non-NODAT

Demographic and biological characteristics	*HC (n=100)*	*NODAT (n=80)*	*Non-NODAT (n=80)*	*P-value*
Age (years) (Mean±SD)	42.56±7.27	39.83±10.21	34.78±8.59	0.143*, <0.0001** 0.0009***
Gender (M/F)	34 (34%)	63 (78.75%)	67 (83.75%)	0.0001+, ++ 0.418+++
	66 (66%)	17 (21.25%)	13 (16.25%)	
BMI (kg/m^2) (Mean±SD)	24.00±1.93	23.31±3.03	23.69±2.98	0.279*, 0.725**
				0.411***

M/F- Male/Female, BMI: Body Mass Index, NODAT: New-onset diabetes after transplantation, HC: Healthy Controls, t-test; + χ^2 test; *Mean±SD independent samples, *NODAT versus HC; **Non-NODAT versus HC; ***NODAT versus Non-NODAT, +NODAT versus HC; ++Non-NODAT versus HC; +++NODAT versus Non-NODAT

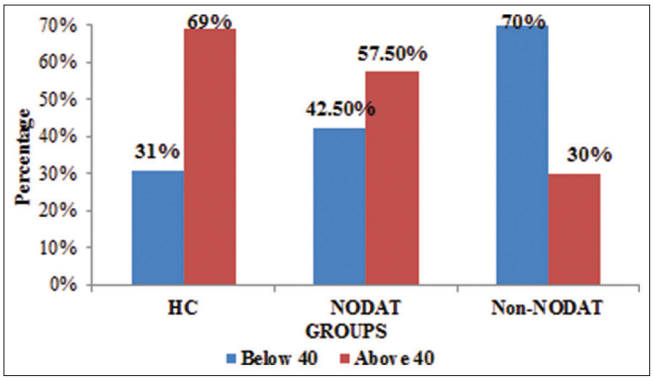

Figure 1: Age-wise distribution in HC, NODAT, and non-NODAT. NODAT: New-onset of diabetes after transplantation, HC: Healthy Controls

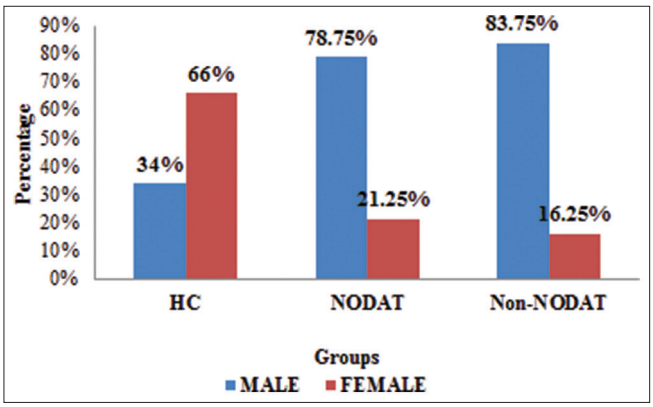

Figure 2: Gender-wise distribution in HC, NODAT, and non-NODAT. HC: Healthy controls, NODAT: New-onset of diabetes after transplantation

HSD revealed that mean scores of the parameters were significantly different among NODAT, non-NODAT, and HC [Table 3].

MLR in HC, NODAT and Non-NODAT

In the present study, MLR analysis was carried out in HC, NODAT, and non-NODAT as outcome variables and age, gender, BMI, biochemical parameters such as creatinine, urea, uric acid, sodium, potassium, chloride, calcium, and phosphorus as predictor variables. In NODAT versus HC, age, gender (male), creatinine, and urea significantly predicted the outcome at $P < 0.013$, $P < 0.0001$, $P < 0.0001$, and $P < 0.015$, respectively. However, BMI, uric acid, sodium, potassium, chloride, calcium, and phosphorus did not predict the outcome significantly. In non-NODAT versus HC, age, gender (male), creatinine, and urea significantly predicted the outcome at $P < 0.0001$, $P < 0.0001$, $P < 0.0001$, and $P < 0.023$, respectively. On the other hand, BMI, uric acid, sodium, potassium, chloride, calcium, and phosphorus did not predict the outcome significantly [Table 4].

ROC Analysis

ROC curve analysis in NODAT and HC

The ROC curves were plotted by computing the sensitivity and specificity of age, gender, BMI, biochemical parameters such as creatinine, urea, uric acid, sodium, potassium, chloride, calcium, and phosphorus in NODAT versus HC. The AUC for creatinine (0.985) and urea (0.918) indicated excellent test, and uric acid (0.620) levels indicated fair test and were observed to be significant at $P < 0.0001$, $P < 0.0001$, and $P < 0.006$. The cutoff value for creatinine was found to be 1.05 mg/dl with sensitivity 93.8% and specificity 92%. The cutoff value for urea was 24.5 mg/dl with sensitivity 91.3% and specificity 65% whereas for uric acid, the cutoff value was observed to be 5.45 mg/dl with sensitivity and specificity of 60% [Table 5 and Figure 5].

Figure 3: Body Mass Index in HC, NODAT, and non-NODAT. NODAT: New-onset of diabetes after transplantation, HC: Healthy controls, UW: Underweight (<18.5), NW: Normal weight (18.5–24.9), OW: Overweight (25–29.9)

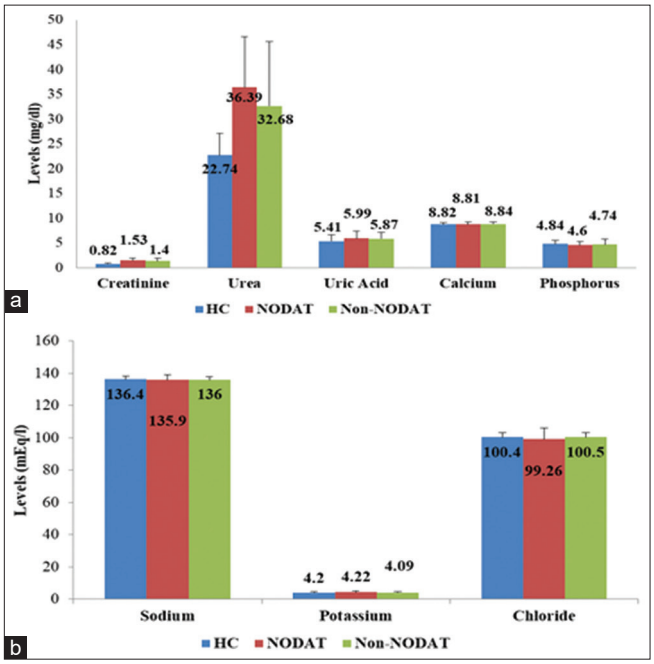

Figure 4: (a) Levels of renal function tests and minerals in Healthy controls, New-onset of diabetes after transplantation, and Non-New-onset of diabetes after transplantation. (b) Levels of electrolytes in Healthy controls, New-onset of diabetes after transplantation, and Non-New-onset of diabetes after transplantation

ROC curve analysis in Non-NODAT and HC

The ROC curves were plotted by computing the sensitivity and specificity of age, gender, BMI, biochemical parameters such as

Table 2: Biochemical parameters in HC, NODAT, and Non-NODAT in serum

Biochemical parameters	*HC (n=100) Mean±SD*	*NODAT (n=80) Mean±SD*	*Non-NODAT (n=80) Mean±SD*	*P-value*
Creatinine (mg/dl)	0.82±0.15	1.53±0.43	1.40±0.58	<0.0001*,** 0.026***
Urea (mg/dl)	22.74±4.41	36.39±10.24	32.68±12.92	<0.0001*,** 0.0009***
Uric acid (mg/dl)	5.41±1.28	5.99±1.36	5.87±1.31	0.006*, 0.014**, 0.79***
Sodium (mEq/l)	136.4±1.90	135.9±3.09	136.0±2.03	0.008*, 0.165**, 0.227***
Potassium (mEq/l)	4.20±0.46	4.22±0.73	4.09±0.54	0.528*, 0.146**, 0.489***
Chloride (mEq/l)	100.4±2.76	99.26±6.74	100.5±2.61	0.220*, 0.799**, 0.164***
Calcium (mg/dl)	8.82±0.33	8.81±0.46	8.84±0.42	0.656*, 0.330**, 0.992***
Phosphorus (mg/dl)	4.84±0.70	4.60±0.69	4.74±1.04	0.029*, 0.078**,0.859***

*Mean±SD independent samples *t*-test, NODAT: New-onset of diabetes after transplantation, HC: Healthy Controls, *NODAT versus HC, **Non-NODAT versus HC, ***NODAT versus Non-NODAT

Table 3: One-way ANOVA for demographic features and biochemical parameters in HC, NODAT, and non-NODAT

Variables	*Sum of Squares (SS)*	*df*	*Mean square (MS)*	*F*	*P-value*
Age					
Between Groups	2722.722	2	1361.361	18.122	0.0001*
Within Groups	19306.140	257	75.121		
Gender					
Between Groups	13.26	2	6.63	36.244	0.0001*
Within Groups	47.03	257	0.183		
BMI					
Between Groups	20.962	2	10.481	1.501	0.225
Within Groups	1794.639	257	6.983		
Creatinine					
Between Groups	26.218	2	13.109	78.071	0.0001*
Within Groups	43.153	257	0.168		
Urea					
Between Groups	9107.219	2	4553.609	50.051	0.0001*
Within Groups	23381.778	257	90.980		
Uric acid					
Between Groups	17.062	2	8.531	4.937	0.008*
Within Groups	444.123	257	1.728		
Sodium					
Between Groups	12.548	2	6.274	1.123	0.327
Within Groups	1435.740	257	5.587		
Potassium					
Between Groups	0.700	2	0.350	1.042	0.354
Within Groups	86.318	257	0.336		
Chloride					
Between Groups	72.649	2	36.324	1.913	0.150
Within Groups	4880.598	257	18.991		
Calcium					
Between Groups	0.043	2	0.021	0.135	0.874
Within Groups	40.813	257	0.159		
Phosphorus					
Between Groups	2.600	2	1.300	1.947	0.145
Within Groups	171.630	257	0.668		

HC: Healthy controls, NODAT: New-onset of diabetes after transplantation, df: degree of freedom; **P*<0.05 was accounted for significant

creatinine, urea, uric acid, sodium, potassium, chloride, calcium, and phosphorus in non-NODAT versus HC. The AUC for creatinine (0.871) indicated good test, urea (0.797) and uric acid (0.607) levels indicated fair test and were observed to be significant at $P < 0.0001$, $P < 0.0001$, and $P < 0.014$. The cutoff value for creatinine was found to be 0.85 mg/dl with sensitivity 82.5% and specificity 64%. The cutoff value for urea was 24.5 mg/dl with sensitivity 75% and specificity 65%, whereas for uric acid, the cutoff value was observed to be 5.65 mg/dl with sensitivity 53.8% and specificity of 65% [Table 6 and Figure 6].

Long-term Outcome, Graft Survival, and Patients' Survival

The renal transplant recipients were followed for 5 years (60 months) to determine the influence of NODAT on the long-term outcome, graft, and patient survival. The renal graft function and the long-term outcome were studied in 80 each of NODAT and non-NODAT subjects by comparing serum creatinine levels and urinary CrCl at 0 month (start of the study) and 60 months (termination of the study).

The mean creatinine levels in serum were 1.53 ± 0.43 and 1.40 ± 0.58 mg/dl at 0 month, whereas at 5 years (60 months), it was found to be 1.71 ± 0.52 and 1.52 ± 0.56 mg/dl in NODAT and non-NODAT, respectively. Significance was observed at 0 month as well as 60 months at $P < 0.0264$ and $P < 0.0157$ in NODAT compared to Non-NODAT. Significance was also observed in NODAT at 0 month versus 60 months at $P < 0.0288$ whereas in non-NODAT, the serum creatinine levels were comparable between both the groups at 0 month versus 60 months [Figure 7].

The urinary CrCl was found to be significant at 0 month in NODAT and non-NODAT (60.36 ± 18.39 versus 76.69 ± 33.64 ml/min) at $P < 0.0029$. The urinary CrCl at 5 years

Table 4: Multinomial logistic regression analysis in HC, NODAT, and non-NODAT as outcome variables and age, gender, BMI, and biochemical parameters such as creatinine, urea, uric acid, sodium, potassium, chloride, calcium, and phosphorus as predictor variables

Group	*B*	*Std. error*	*Sig*	*Exp (B)*	*95% confidence interval for Exp (B)*	
					Lower bound	*Upper bound*
NODAT versus HC						
Intercept	−10.565	18.539	0.569			
Age	−0.086	0.034	0.012	0.918	0.859	0.981
BMI	−0.213	0.112	0.056	0.808	0.649	1.006
Creatinine	6.834	1.454	0.000	929.146	53.716	16.71.668
Urea	0.143	0.063	0.023	1.153	1.020	1.304
Uric acid	−0.017	0.232	0.942	0.983	0.624	1.550
Sodium	0.033	0.123	0.789	1.033	0.813	1.314
Potassium	−0.175	0.476	0.713	0.839	0.330	2.133
Chloride	−0.089	0.090	0.325	0.915	0.767	1.092
Calcium	1.429	0.856	0.095	4.176	0.779	22.375
Phosphorus	−0.144	0.354	0.684	0.866	0.432	1.733
[Gender=0]	2.063	0.594	0.001	7.873	2.459	25.205
[Gender=1]	0[b]	.	.	.	.	.
Non-NODAT versus HC						
Intercept	−10.919	18.485	0.555			
Age	−0.148	0.034	0.000	0.863	0.807	0.922
BMI	−0.134	0.111	0.227	0.874	0.703	1.087
Creatinine	6.473	1.463	0.000	647.287	36.801	11385.171
Urea	0.125	0.063	0.047	1.133	1.002	1.282
Uric acid	0.045	0.230	0.844	1.046	0.666	1.643
Sodium	−0.036	0.123	0.773	0.965	0.758	1.229
Potassium	−0.402	0.485	0.406	0.669	0.259	1.729
Chloride	0.025	0.092	0.783	1.026	0.856	1.229
Calcium	1.389	0.852	0.103	4.009	0.755	21.288
Phosphorus	−0.087	0.346	0.801	0.917	0.466	1.804
[Gender=0]	2.654	0.612	0.000	14.218	4.287	47.155
[Gender=1]	0[b]	.	.		.	.

Table 5: Area under curve, cutoff values, sensitivity, and specificity in NODAT versus HC

Variables	*Area*	*Std. Error*	*Asymp-totic Sig.*	*Asymptotic 95% CI*		*Cut off value*	*Sensitivity (%)*	*Specificity (%)*
				Lower Bound	*Upper Bound*			
Age	0.436	0.045	0.143	0.349	0.524	44.5	40	51
BMI	0.453	0.045	0.279	0.364	0.542	24.3	45	59
Creatinine	0.985	0.008	0.0001	0.970	1.000	1.05	93.8	92
Urea	0.918	0.020	0.0001	0.879	0.957	24.5	91.3	65
Uric acid	0.620	0.042	0.006	0.538	0.702	5.45	60	60
Sodium	0.387	0.043	0.009	0.302	0.471	136.5	35	53
Potassium	0.473	0.045	0.528	0.385	0.561	4.25	42.5	58
Chloride	0.447	0.044	0.223	0.361	0.534	100.5	45	43
Calcium	0.519	0.047	0.656	0.428	0.611	8.95	50	68
Phosphorus	0.596	0.042	0.028	0.513	0.679	4.65	58.8	49

AUC: Area under the curve, NODAT: New-onset of diabetes after transplantation, HC: Healthy controls

was 58.48 ± 16.20 and 73.06 ± 26.67 ml/min in NODAT and non-NODAT, respectively, and was found to be significant at $P < 0.0006$. Significance was also observed in NODAT at 0 month and 60 months at $P < 0.0047$, whereas in non-NODAT, the urinary CrCl was comparable between both the groups at 0 month versus 60 months [Figure 8].

At the start of the study, 4 (5%) subjects in NODAT were infected with HCV whereas in non-NODAT, 3 (3.75%) individuals were infected with HCV and significance was not observed between the groups. Both in NODAT and non-NODAT, only 1 (1.5%) recipient was found to be positive with Hepatitis B surface antigens with no statistical significance. Only 1 (1.5%) patient in NODAT was infected with tuberculosis. During the course of study, these patients were treated and tested negative for the above mentioned viral and bacterial infections [Table 7].

In NODAT, delayed graft function was noted in 2 (2.5%) recipients, which could be explained by the fact that they received cadaveric transplants. The patient survival and graft survival rates at 5 years (60 months) were 97.5% in NODAT and 100% in non-NODAT group, with no significance observed between both the groups as demonstrated in Figure 9. The graft loss was 2.5% in NODAT, which was mainly due to death of 2 recipients (2.5%) in NODAT, whereas in non-NODAT, there were no graft loss and patient loss. The reasons for death in NODAT subject were mainly due to pneumonia and cardiac arrest, apart from development of diabetes after renal transplantation [Table 8].

Discussion

Age is considered as an important risk factor for NODAT, especially in patients >40 years, as described in various studies.[30-32] We too observed that the patients who developed diabetes were comparatively older (39.83 ± 10.21) than the patients who did not develop diabetes after renal transplantation (34.78 ± 8.59) which

Table 6: Area under curve, cutoff values, sensitivity, and specificity in non-NODAT versus HC

Variables	*Area*	*Std. Error*	*Asymp-totic Sig.*	*Asymptotic 95% CI*		*Cut off value*	*Sensitivity (%)*	*Specificity (%)*
				Lower Bound	*Upper Bound*			
Age	0.243	0.037	0.0001	0.170	0.315	42.5	20	44
BMI	0.485	0.046	0.724	0.395	0.574	24.3	46.3	59
Creatinine	0.871	0.030	0.0001	0.813	0.929	0.85	82.5	64
Urea	0.797	0.033	0.0001	0.732	0.862	24.5	75	65
Uric acid	0.607	0.043	0.014	0.523	0.690	5.65	53.8	65
Sodium	0.441	0.043	0.172	0.356	0.526	136.5	41.3	53
Potassium	0.437	0.044	0.146	0.352	0.522	4.15	43.5	50
Chloride	0.511	0.043	0.800	0.426	0.596	101.5	40	61
Calcium	0.542	0.044	0.332	0.455	0.629	8.85	55	50
Phosphorus	0.533	0.044	0.453	0.453	0.618	4.55	52.5	48

AUC: Area under the curve, NODAT: New-onset of diabetes after transplantation, HC: Healthy controls

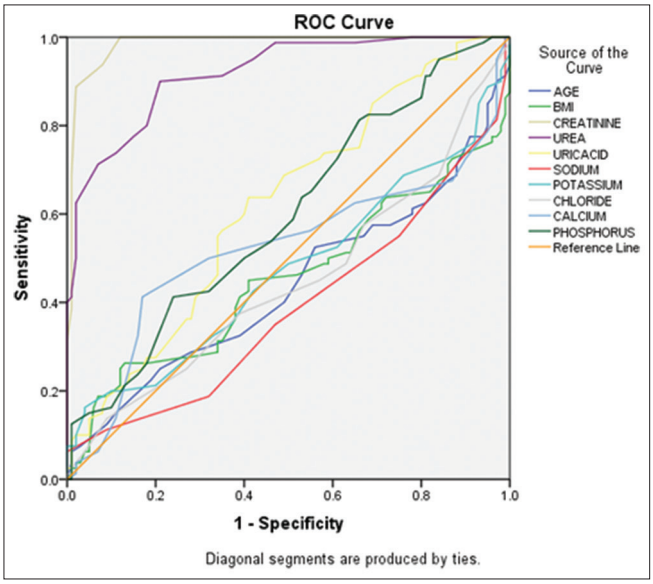

Figure 5: Receiver Operating Characteristic curve analysis in New-onset of diabetes after transplantation versus Healthy controls

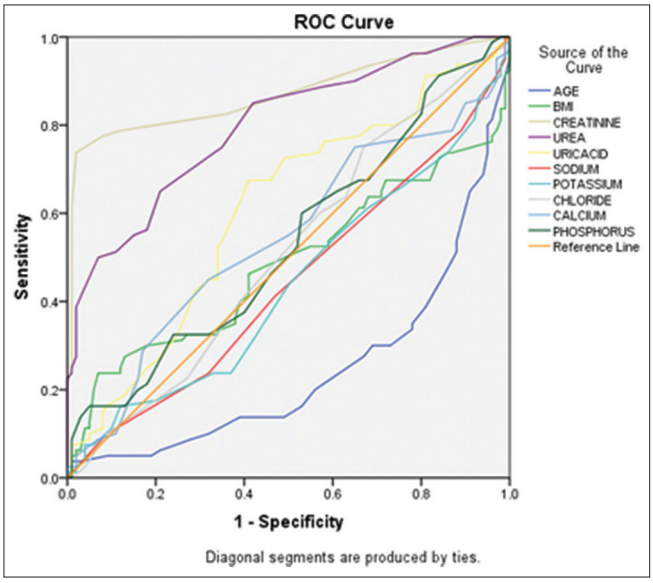

Figure 6: Receiver Operating Characteristic curve analysis in Non-New-onset of diabetes after transplantation versus Healthy controls

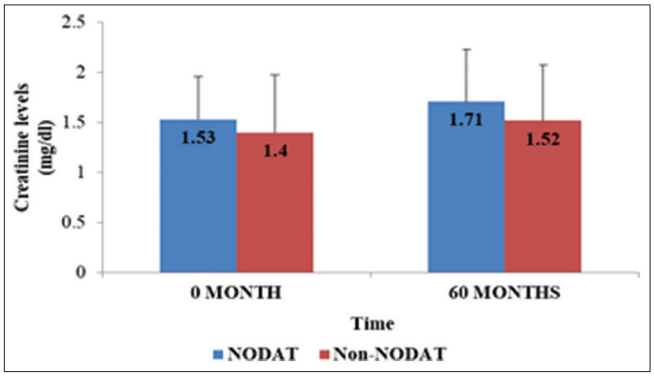

Figure 7: Creatinine levels in NODAT and Non-NODAT at 0 month and 60 months. HC: Healthy controls, NODAT: New-onset of diabetes after renal transplantation

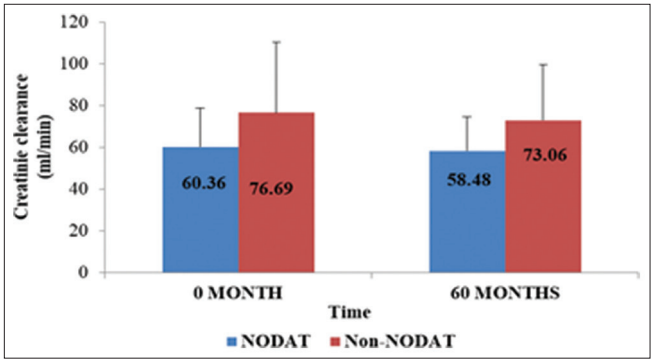

Figure 8: Urinary creatinine clearance in New-onset of diabetes after renal transplantation and Non-New-onset of diabetes after renal transplantation at 0 month and 60 months

was consistent with various studies in different population. Similar results were observed in NODAT (39.3 ± 13.4) versus non-NODAT (33.9 ± 11.8) in Malaysian population.[33] Older age was observed as the risk factor for NODAT in populations of Brazil,[34] Turkey,[35] Portugal,[36] South Africa,[37] and Singapore.[38] Cosio *et al.*[6] reported that there was 2.9 times increased risk in individuals undergoing renal transplantation, of age >45 years than younger patients. Gourishankar *et al.*[39] have reported 1.5 times more risk for developing NODAT for every decade increase in life. Hjelmesaeth *et al.*[40] observed the older age to be a significant factor for reduced β-cell function post-kidney transplantation. They demonstrated that increasing age was independently and strongly related to reduced insulin secretory phase. It is also possible that older patients are more likely to be susceptible than younger patients to same doses of immunosuppressive drugs.

Table 7: Rate of infections in NODAT and Non-NODAT at 0 month

	NODAT (n=80) Frequency (%)	*Non-NODAT (n=80) Frequency (%)*	*P-value*
HCV	4 (5)	3 (3.75)	1.000+
HBsAg	1 (1.5)	1 (1.5)	1.000+
TB	1 (1.5)	-	

HC: Healthy controls, NODAT: New-onset of diabetes after renal transplantation, HCV: Hepatitis C Virus, HBsAg: Hepatitis B surface antigen, TB: Tuberculosis, +χ^2 test

Table 8: Efficacy end points at 5 years in NODAT and Non-NODAT

	NODAT (n=80) Frequency (%)	*Non-NODAT (n=80) Frequency (%)*	*P-value*
Delayed graft function	2 (2.5)	-	
Graft loss	2 (2.5)	-	
Death	2 (2.5)	-	
Graft survival	78 (97.5)	80 (100)	0.497+
Patient survival	78 (97.5)	80 (100)	0.497+

HC: Healthy controls, NODAT: New-onset of diabetes after renal transplantation, +χ^2 test

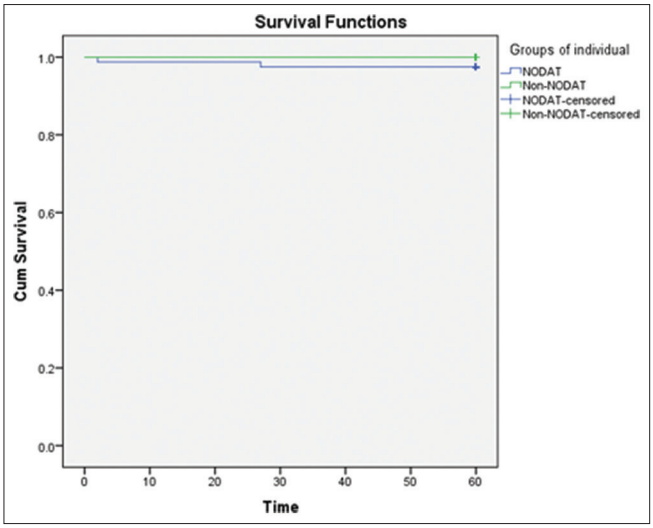

Figure 9: Kaplan-Meier survival curve analysis in NODAT and Non-NODAT. HC: Healthy controls, NODAT: New-onset of diabetes after renal transplantation

We have observed higher frequencies of males than females in the KTRs at our center, though significance in gender was not observed in NODAT as compared to non-NODAT groups, which is similar to the study in Malaysian population.[33] Similarly, different studies among Indian population, that is, from Kerala,[41] Kashmir,[42] Kolkata,[43] and Uttar Pradesh[33] have shown higher percentage of males than females in KTRs and they did not observe statistically sex difference. Kasiske *et al*.[5] and Shah *et al*.[44] have observed males to be at greater risk for NODAT development as compared to females. In contrast to our study, it has been demonstrated in Turkish population that the percentage of females were higher than males in NODAT whereas in non-NODAT group, they observed higher frequency of males as compared to females.[35]

We have not observed any significance in the BMI in NODAT compared to non-NODAT groups. Similar observations were reported in populations of Malaysia[33] and Singapore.[38] In Indian studies from Kerala[41] and Kashmir,[42] significance was not found in BMI between NODAT versus non-NODAT groups which is similar to the present study. The previous studies have reported that NODAT is associated with obesity.[45] Bonato *et al*.[46] reported that obese patients or overweight patients to be associated with NODAT development. Choudhury *et al*.[43] observed increased pre-transplant BMI in NODAT than non-NODAT patients. A study in Turkish population has revealed that obese or overweight patients were at higher risk for NODAT development.[35] The variation between our study and others study could be elucidated by the fact that none of the recipients at our center was obese (BMI>30 kg/m^2). Most of them were observed to be of normal weight.

Jusufovics *et al*.[47] have found higher creatinine levels in patients with T2DM than patients without diabetes. Another study in Malaysian population did not find significant difference in creatinine levels between NODAT versus non-NODAT.[33] Some studies have observed higher serum creatinine levels in NODAT as compared to patients who did not have post 5 years of transplantation.[48] We did not observe significance in NODAT compared to non-NODAT, though significance was observed between NODAT and healthy controls. A study from Hungary has reported higher creatinine levels in NODAT than healthy controls, though they did not observe significance in urea levels between both groups,[49] whereas we found higher urea levels in NODAT as compared to healthy controls. The previous researchers reported correlation of higher serum uric acid levels with diabetes,[16,17,50] Similarly, we observed higher serum uric acid levels in NODAT than HC. A follow-up study for 16 years on Japanese population indicated negative correlation between uric acid and increased risk of T2DM.[19] A study from India by Modi *et al*.[18] reported no significant correlation of serum uric acid with blood sugar levels in diabetics patients. Some studies have observed higher serum uric acid levels in patients with pre-diabetic condition and early T2DM than those without diabetes.[51,52] An Indian study from Maharashtra has found significant association between serum creatinine and uric acid levels and observed elevated serum creatinine levels with the increase in uric acid levels. They also found positive association between uric acid and fasting blood glucose levels (P = 0.004). The same study has also reported higher blood urea in T2DM patients.[53]

Diabetes is associated with dysnatremias (hyponatremia-low sodium levels and hypernatremia-high sodium levels) through many different mechanisms.[54] Hyperglycemia enhances the osmolality of serum which expels water outside the cells resulting in depletion of sodium levels (Na^+) in serum by dilution.[55] We observed significantly decreased serum sodium levels in NODAT as compared to HC, but there was no change in the levels when compared to non-NODAT. In a study consisting of 5179 community subjects with average age of 55 years or more, hyponatremia was observed in DM subjects.[54] Hypernatremia is related to endocrine dysfunction and also it has been noted that in humans and animals, hypernatremia, and hyperosmolarity are linked with impairment of both glucagon-dependent glucose release and insulin-mediated glucose metabolism.[56,57]

Some studies have shown the incidence of hyperkalemia (increased potassium levels) in DM patients as compared to

general population. In general, healthy diabetic diet is usually rich in potassium and low in sodium, which contributes to the hyperkalemia occurrence in susceptible individuals.[58,59] However, the typical cause of chronic hyperkalemia in diabetes is the reduction in tubular secretion of potassium due to hyporeninemic hypoaldosteronism syndrome.[60] The present study observed increased levels of potassium in the serum of NODAT subjects as compared to non-NODAT and HC, though significance was not observed between the groups. It is also observed that exogenous insulin may induce hypokalemia by promoting the potassium entry into hepatic cells and skeletal muscles through increase in the activity of the Na^+- K^+ -ATPase pump.[61]

Increased levels of chloride are observed in T2DM patients which occurs due to diabetic ketoacidosis. Reduction in pH of blood is triggered by ketoacids leading to disturbance in the acid-base balance which causes increase in the chloride levels. In the present study, slightly decreased serum levels of chloride were observed in NODAT as compared to non-NODAT and HC; however, significance was not observed. In a study from Kancheepuram District among diabetic individuals, sodium levels were observed to be lower as compared to controls whereas, potassium and chloride were higher as compared to controls, in which potassium levels were found to be significant.[62]

Homeostasis of calcium exerts its influence on insulin secretion and insulin resistance.[63] In a study from Baghdad comprising 30 subjects of 30–70 years of age, increased serum calcium levels with substantial decreased parathyroid levels were observed.[64] Another study from India[65] and North Sudan[66] demonstrated significant reduced serum calcium levels in T2DM patients as compared to healthy controls. In contrast, Chen *et al*.[67] showed an increased risk of T2DM in subjects having elevated levels of calcium in serum. We did not observe any significant change in the serum calcium levels in NODAT versus HC; however, levels were reduced in comparison with non-NODAT, although it did not reach to the significance level.

The present study showed decreased serum levels of phosphorus in NODAT as compared to HC as well as non-NODAT, however, significance was observed between NODAT versus HC. A study from Punjab has shown reduced serum levels of phosphorus in T2DM patients as compared to healthy controls.[68] Hamad *et al*.[69] in their study among Sudanese population in Khartoum State demonstrated significant reduction in levels of phosphorus in serum in T2DM patients than controls; however, they did not observe a change in serum calcium levels between diabetic and non-diabetic patients. In a study from Kashmir among renal transplant recipients, higher means levels of calcium and lower mean levels of phosphorus have been reported in NODAT subjects than normal individuals.[42]

Conclusion

The present study revealed that older age (above 40) to be significant factor for the development of NODAT. Higher levels of creatinine, urea, and uric acid in serum might be associated with the development of NODAT and could also be the important markers for the assessment of kidney function in renal transplant recipients. Among these, creatinine was found to be the best marker for the assessment of kidney function. Hyponatremia (low Na^+ levels) and hypophosphatemia (low phosphate levels) could also be risk factors for the development of NODAT.

The reduced CrCl at 60 months than 0 month in NODAT subjects indicated reduced functioning of the graft as compared to non-NODAT subjects. Even though new-onset diabetes had adverse impact on renal transplant recipients, overall survival rate was not reduced much and the 5-year survival rate of the patient/graft was found to be 97.5%.

Credit Authorship Contribution Statement

Shazia Ahmad: Performed the experiments, data analysis, writing, editing, and revising the manuscript. Kesiraju Sailaja: conceptualization, designing of experiments, and resources. Penagaluru Pardhanandana Reddy: conceptualization, designing of experiments, and resources. Sumanlatha Gaddam: Supervision, conceptualization and designing of experiments, resources, and revising the manuscript. All authors read and approved the final manuscript.

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